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The Changing Face of the Immunoassay Landscape for Soluble Target Engagement Biomarker Quantification

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Overview

1. Soluble Target Engagement Biomarkers and their Importance
2. Common Technologies for Quantification
3. Considerations with Case Studies
4. Summary

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Biomarkers

"a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."

National Institute of Health

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Soluble Target Engagement Biomarkers (STEMs) are a Pivotal Tool in Drug Development

- Preclinical and clinical development of a drug molecule is a lengthy process that requires well-designed studies with carefully chosen and defined outputs
- Many biotherapeutic drugs are designed to target a specific receptor or analyte
- These targets may be located at the cell membrane and are therefore not easily accessible for analysis
- Alternatively, they may be in the circulation as natural ligands or as a result of membrane receptor shedding
- Where such a soluble form exists, the target itself can be utilised as a **biomarker of target engagement**

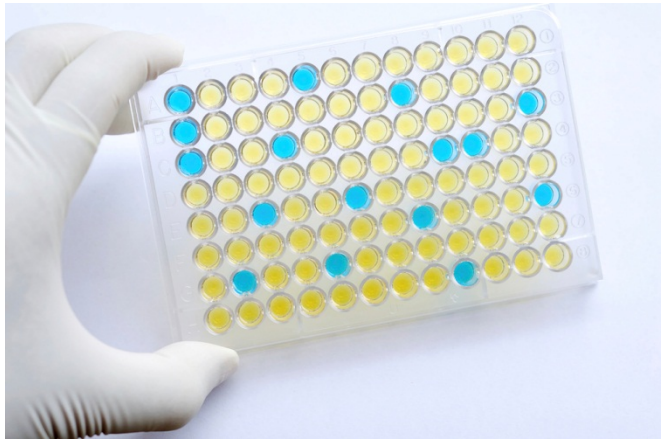
Target Engagement Biomarkers are Important to Demonstrate Efficacy and Aid Dose Selection

- Demonstrate **binding** of the biotherapeutic molecule to its target
- The potential to determine this soluble form in easily accessible biological fluids, such as serum or plasma, can be particularly informative to assess the interdependent **relationship of drug and target**
- Quantification of the required amount of target to have a **disease modifying effect**
  - Particular importance for first in class molecules
- **Allometric scaling** across species
- **Optimal duration** of impacting the target and **subsequent repeat administration** regimens can be determined
- In the case of many biologics, monitoring the loss of drug efficacy due to **immunogenicity**

Biomarker Assays Present Challenges not Found in Pharmacokinetic (PK) Assays

Attribute	PK Assay for Drug	PD Assay for Biomarker
Molecule detected	• Drug	• Antigen/ligand
Quantitative	• Considered fully quantitative	• Generally not fully quantitative
Sensitivity	• µg/mL to ng/mL	• ng/mL to pg/mL (or less)
Capture reagent	• Anti-IgG antibody • Anti-idiotype antibody • Target antigen	• Anti-analyte antibody • Soluble receptor
Reference calibrator	• Drug itself • Well characterised	• Recombinant form of antigen • May change vendor-to-vendor or lot-to-lot • Purified endogenous form
Analyte stability	• Generally stable through multiple freeze-thaw cycles	• May be prone to analyte instability, particularly on initial freezing or repeated freeze-thaw cycles
Calibrator matrix	• Same as sample at minimal required dilution	• Substitute matrix • Matrix depleted of analyte of interest
Minimal required dilution	• Often higher than PD assays to overcome interference	• Kept at a minimum to avoid disrupting equilibrium
Interfering substances	• Soluble target • Anti-drug antibodies • Endogenous IgG	• Anti-drug antibodies • Soluble receptor • Binding proteins
Kit availability	• Universal IgG assays may be developed or available as a kit	• Specific analytes • Singleplex or multiplexed
Regulation	• Regulated	• ‘Fit for purpose’

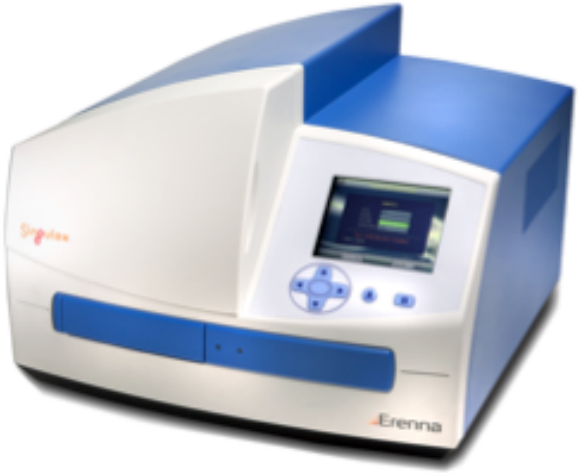
Immunoassays: The ELISA



- Well established technique
- Relatively cheap
- No fancy equipment, non-vendor reliance
- Availability of various labelling chemistries
- Widely available in bioanalytical labs across the globe
- Easy to transfer within company and to CROs
- Other technologies have moved into the space that ELISA once dominated .....
  - Sensitivity
  - Assay time
  - Multiplexing
  - Smaller sample volume

Other Platforms are Replacing ELISA

Cirascan

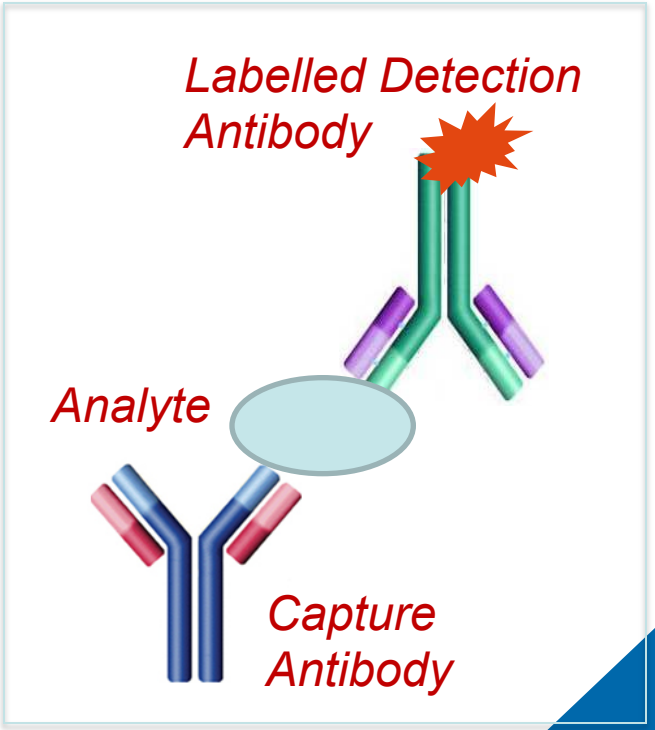


Examples of Common Platforms Taking the Place of ELISA: One Size Does Not Fit All

Technology	Advantages	Disadvantages
MSD	• Sensitive • Large dynamic range • Decreased sample volume compared to ELISA • Established technology • Plate reading time is short, allowing multiple users • Machine reliability • CRO availability • Kits	• Vendor specific • Expensive compared with ELISA • Requires significant analyst time • Multiplexing of customised assays requires plates to be manufactured by MSD • If direct conjugation used, labelling is required
Gyrolab	• Quick assay development time • Semi-automated • Low sample volume • Gyrolab Viewer to assess column binding • User friendly software • Kits now becoming available	• Vendor specific • Expensive compared with ELISA • System training required • One machine, one analyst • Needle carryover can be an issue • Specific labelling if commercially conjugated antibodies are not available
Singulex	• Single molecule counting • Extremely sensitive • Potential for sub pg/mL detection • Kits	• Less common technology • CRO availability • Expensive compared to ELISA and in some cases MSD and Gyrolab • System and bead conjugation training required

Case Study 1: Significant Levels of Endogenous Analyte can Interfere with Assay Sensitivity

- Free PD assay to detect analyte not bound to therapeutic drug molecule
- Aim: 99% suppression of soluble target on administration of therapeutic monoclonal antibody
- Circulating levels approximately 3 ng/mL in disease state human plasma
- 30 pg/mL assay sensitivity required
- Normal human plasma used as calibrator matrix



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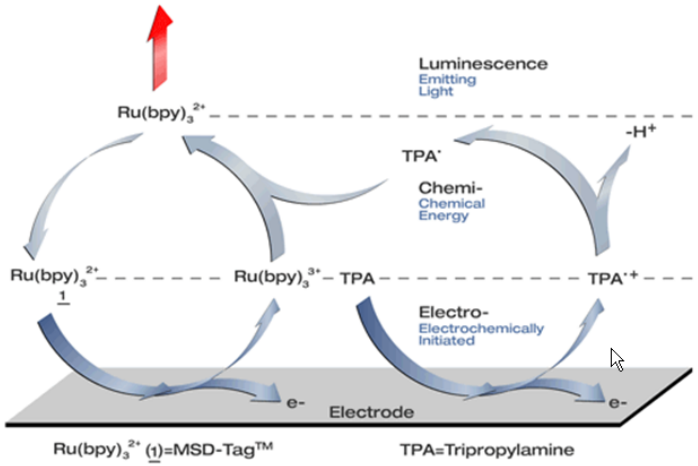
Meso Scale Discovery



- Platform Choice:
  - Increased dynamic range over ELISA
  - Electrochemiluminescence
  - Increased binding surface
  - Only molecules captured at surface would be detected
  - Sample volume was not problematic
- Remove endogenous levels of analyte from the matrix
- Another species matrix was not an option due to cross reactivity of assay antibodies
- Treated matrix with sepharose protein A beads coupled with anti-target antibody

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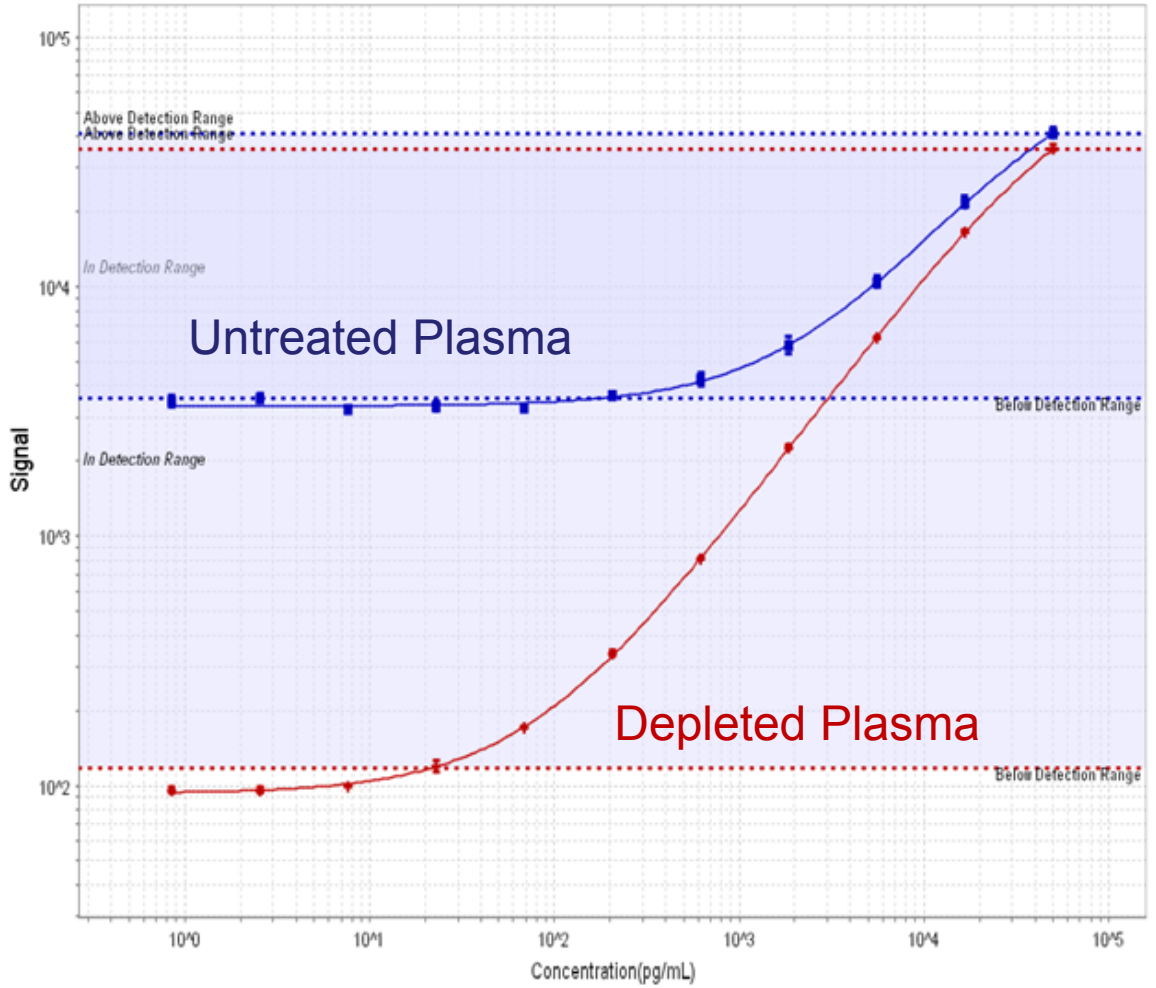
Depletion of Endogenous Analyte can Increase Sensitivity



1000 pg/mL verses 30 pg/mL lower limit of quantification (LLOQ)

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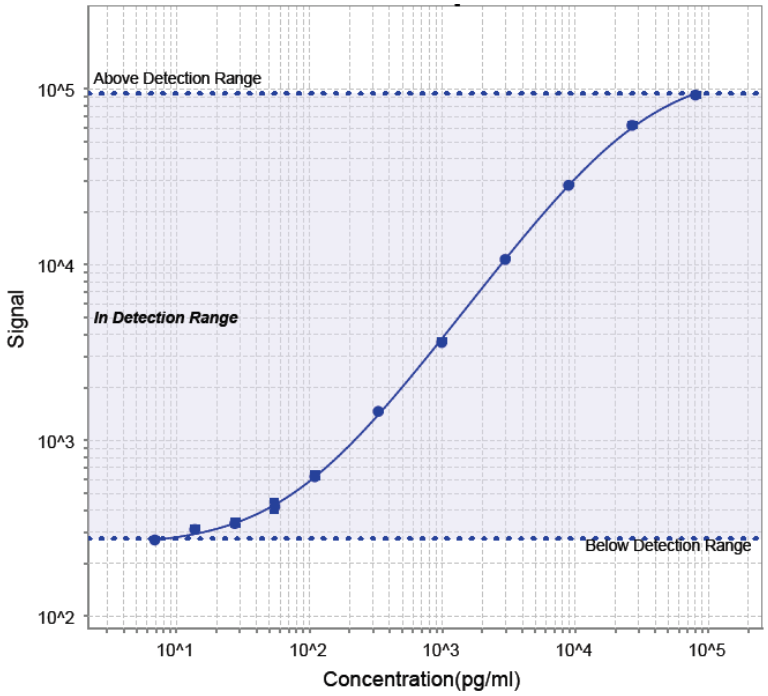
Further Increases of Sensitivity were Achieved by Pre-treating the Plate with MSD Diluent

- Half hour incubation with MSD Diluent in well after capture antibody and blocking steps
- Equal volume of sample added without washing
- Assay protocol continued as normal



Improved LLOQ of 10 pg/mL achieved

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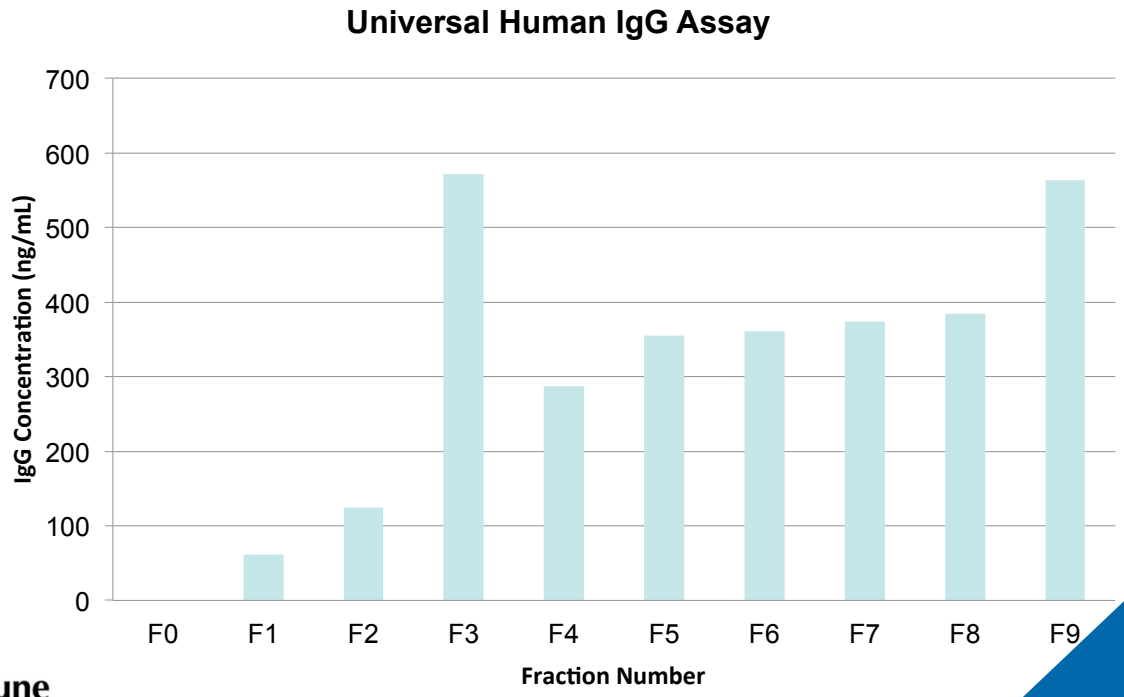
A Word of Caution when Depleting Matrix

- Bear in mind that any amount of free drug that is not conjugated to the beads may appear in your calibrator matrix
- This should be checked otherwise your assay may yield unexpected results



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Case Study 2: Gyrolab™ was used to Apply Automation

- Platform Choice:
  - Mouse assay and therefore sample volume was critical
  - Avoided high sample dilution needed for other 96 well plate based formats
  - Shorter incubation time on the column had the potential to overcome matrix interference seen with longer incubation assays
  - High throughput of 1 hour per CD was advantageous for project time lines

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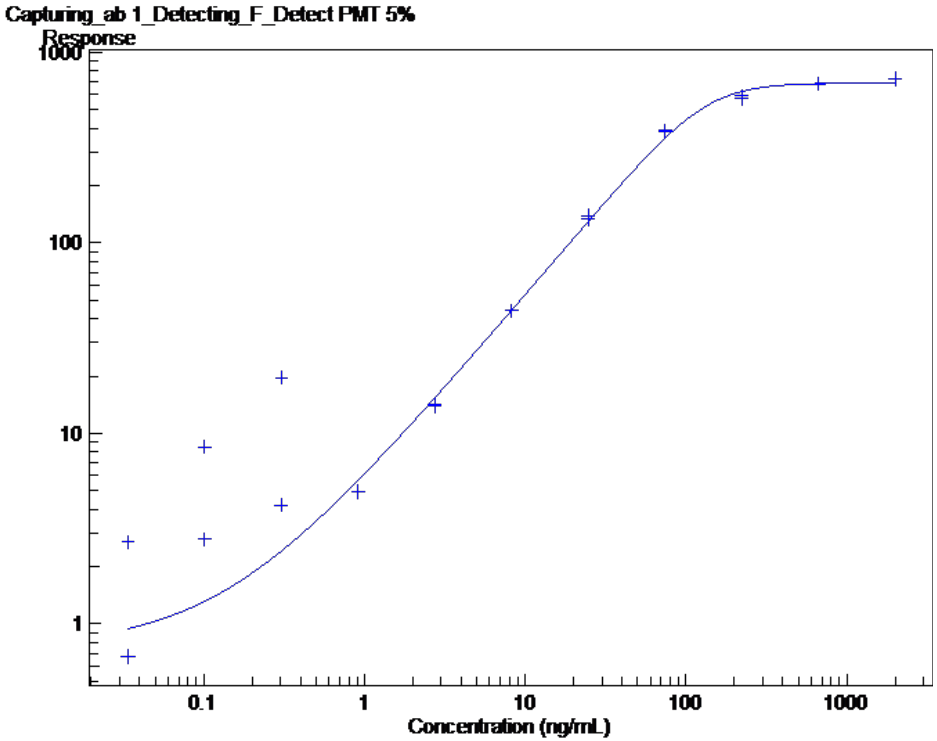
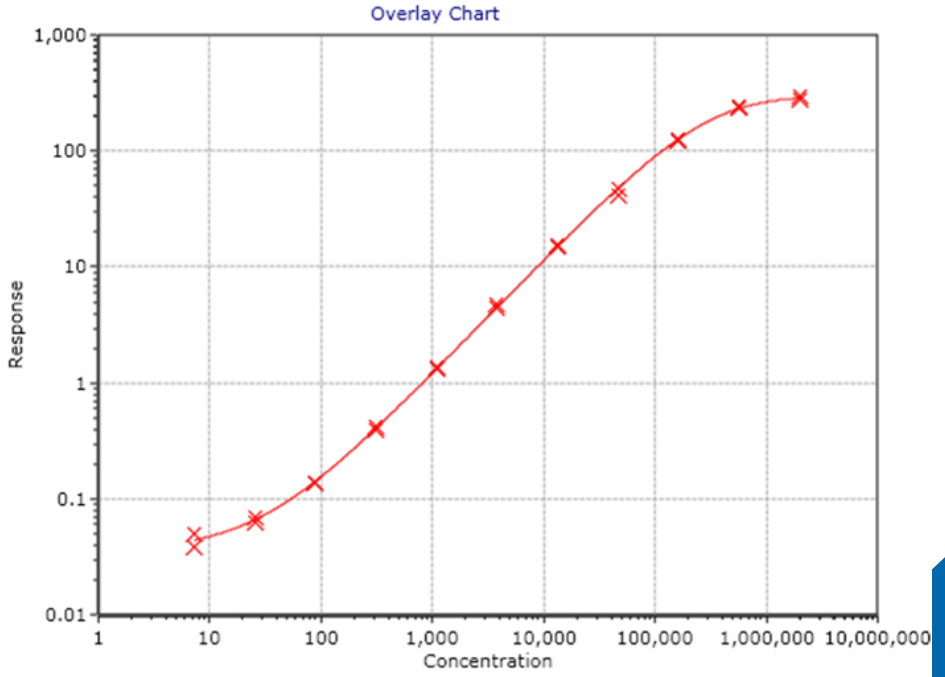
Static Needles in the Gyrolab System can Cause Carryover





- Second replicate higher than first
- Could just be the sensitivity achievable with the assay antibodies

- Rexxip A sample diluent was adjusted to pH 8.5
- Wash solution of 50 mM glycine/0.5% SDS (pH 9.5)
- Reduction of top standard

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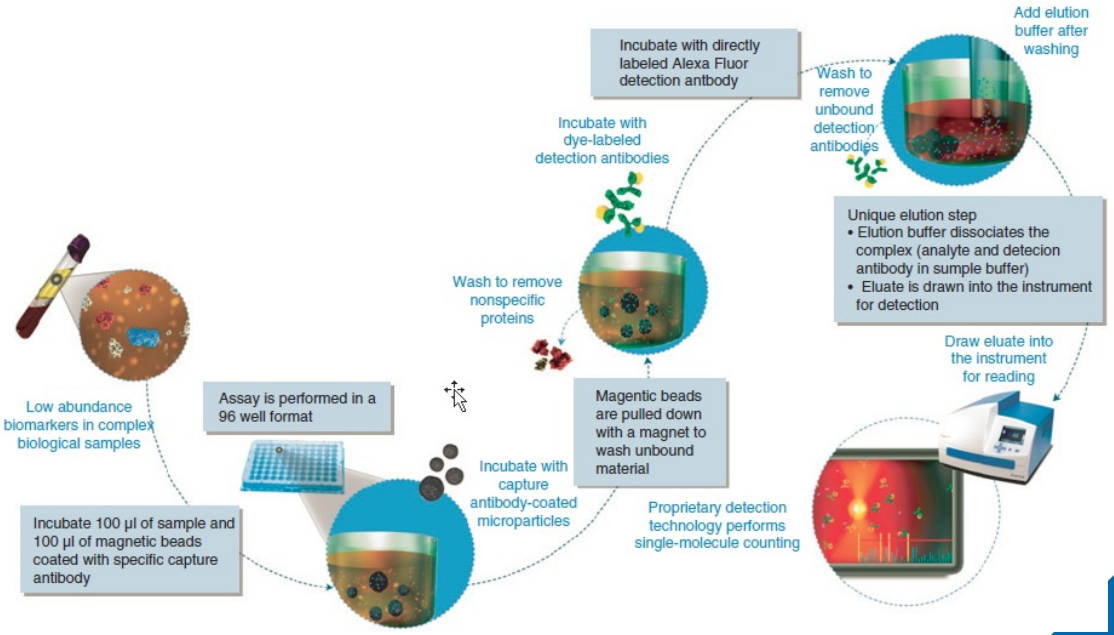
Case Study 3: Singulex Single Molecule Counting

- Platform Choice:
  - Low circulating levels of target
  - MSD assay
    - LLOQ 3.9 pg/mL
  - Gyrolab assay
    - LLOQ 30 pg/mL
  - Commercial samples even in disease state all BLOQ



Assay development at Singulex (prior to platform being available as an open platform) resulted in a LOD of 0.12 pg/mL, LLOQ 0.24 pg/mL

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Commercial Samples all Yielded Positive Results

Sample Number	Replicate 1	Replicate 2	Replicate 3	Mean	Standard Deviation	% CV
1	1.16	1.07	1.20	**1.14**	0.07	5.8
2	1.59	1.50	1.48	**1.52**	0.06	3.8
3	1.45	1.58	1.50	**1.51**	0.07	4.3
4	1.83	1.88	1.53	**1.75**	0.19	10.8
5	1.69	1.45	1.49	**1.54**	0.13	8.3
6	1.50	1.65	1.85	**1.67**	0.18	10.5
7	2.50	1.88	1.98	**2.12**	0.33	15.7
8	1.69	1.95	1.65	**1.76**	0.16	9.2
9	1.44	1.46	1.28	**1.39**	0.10	7.1
10	1.49	1.82	1.26	**1.52**	0.28	18.5
11	1.58	1.33	1.48	**1.46**	0.13	8.6
12	1.94	1.82	1.95	**1.90**	0.07	3.8
13	2.05	2.19	2.24	**2.16**	0.10	4.6
14	1.00	1.20	1.22	**1.14**	0.12	10.7
15	1.28	1.31	1.56	**1.38**	0.15	11.1
16	1.51	1.53	1.36	**1.47**	0.09	6.3
17	1.78	1.71	1.76	**1.75**	0.04	2.1
18	1.60	1.63	1.70	**1.64**	0.05	3.1
19	1.63	1.71	1.90	**1.75**	0.14	7.9
20	1.65	1.76	2.00	**1.80**	0.18	9.9

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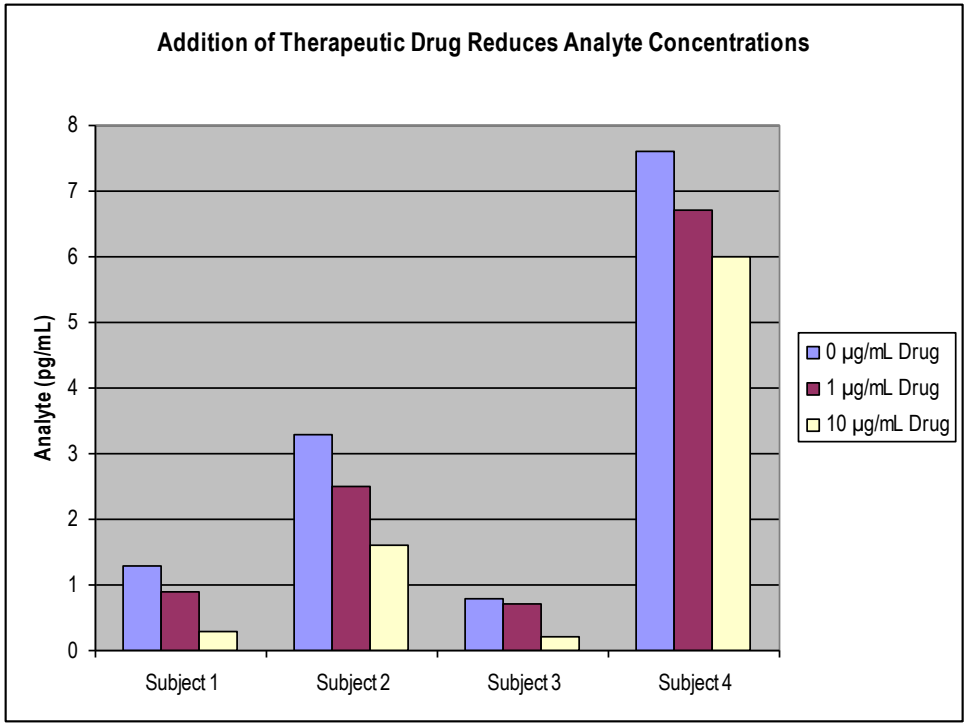
Added Complication: Dimeric Target – When a Free Assay is not Truly Free

- Potential for one subunit of the dimer to bind capture antibody and the other to have drug bound to it
- Often steric hindrance may prevent this occurring
- Assay developed using same antibody for capture and detection



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Summary

- The immunoassay space has evolved dramatically over the last decade, with newer technologies gaining popularity over ELISA
  - Greater sensitivity
  - Increased dynamic range
  - Smaller sample volumes
  - Shorter run times
- Many of these alternative technologies are expensive
  - When used in a multiplex format not only is the sample volume reduced but so is the cost per analyte
- When developing assays for a soluble target engagement biomarker multiplexing is not feasible, and therefore cost can become a major player and may dictate the platform of choice
- Many more factors present issues in this type of immunoassay development, such as the right choice of reagents, minimizing interference from matrix and even endogenous levels of the analyte of interest in the calibrator matrix
- Carefully choice of assay platform may overcome some challenges
- No one size fits all

Questions

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